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Effects of Endogenous Serum Neuregulin-1 β on Morbidity and Mortality in Patients with Heart Failure and Left Ventricular Systolic Dysfunction

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Abstract

Context: Improved left ventricular ejection fraction (LVEF) following administration of recombinant human Neuregulin-1 β (NRG), epidermal growth factor (EGF) involved in cardiomyocyte repair/survival, has been observed in patients with systolic heart failure (HF).

Methods: Serum NRG was measured by ELISA in 248 patients with NYHA class I-IV HF.

Results: NRG exhibited a marginally significant effect on LVEF trajectory over 11 months ($p=0.07$). There is no apparent level of NRG that predicts improved survival.

Conclusion: There is a potential relationship between serum NRG and improved LVEF, indicating the need to investigate the utility of NRG in predicting HF outcomes, including LVEF maintenance.

Keywords

Neuregulin-1; heart failure; left ventricular ejection fraction; left ventricular systolic dysfunction; cardiomyopathy

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Disclosure of Interest Statement

All other authors have no conflicts of interest to disclose.

Introduction

Neuregulin 1 β (NRG) is an epidermal growth factor that activates cardiomyocyte erythroblastic leukemia viral oncogene (ErbB2 and ErbB4) signaling pathways to promote myocyte differentiation and survival, improvement of sarcomeric integrity, and enhances cardiac repair in response to injury (1). NRG activates intercalated disk focal adhesion kinase (FAK) and promotes intermyocyte connections among isolated adult rat ventricular myocytes (2). In the setting of oxidative stress or cytotoxic stimuli, NRG/ErbB signaling protects myocardial cells from apoptosis (3). Furthermore, NRG and phosphorylated ErbB4 levels have been shown to be up-regulated directly following ischemic injury in rats, and deleting NRG in ischemic mouse models resulted in a significant reduction in left ventricular systolic function, further demonstrating its cardioprotective and reparative role (4, 5, 6). The idea that NRG, an active ligand for ErbB2 and ErbB4 receptors on the myocardial cell, may be a cardioactive growth factor in myocyte physiology was first considered clinically when patients undergoing chemotherapy with trastuzumab, a monoclonal antibody that blocks the ErbB2 receptor on cancer cells, were found to be at increased risk of developing left ventricular systolic dysfunction (LVSD) (6, 7). Subsequent studies have found that NRG has the potential to become a biomarker for predicting heart failure (HF) disease severity independent of B-type natriuretic peptide, where increased levels of serum NRG were suggestive of increased compensatory response to stress, and that NRG-1/ErbB signaling is upregulated during early stages of HF (1,8). In addition to its role as a ligand for cardioprotective signaling, NRG also has substantial therapeutic potential. Several placebo-controlled, double-blind, phase I and II clinical trials have found that administration of recombinant human NRG-1 improved cardiac function in patients with LVSD (9, 10, 11). These findings illustrate the need to understand the endogenous human response to NRG, especially in the context of LVSD.

Clinical Significance

- An important question remains as to whether higher serum NRG levels are associated with subsequent improvements in LV systolic function earlier in the course of disease. The purpose of this study is to correlate serum NRG with left ventricular ejection fraction (LVEF) as documented through serial 2-dimensional echocardiography (2D-Echo) and transplant and advanced therapy-free survival. We hypothesize that patients with higher baseline levels of NRG will have improved LVEF in the setting of LVSD.

Materials and Methods

Serum NRG was obtained in a total of 248 patients who were enrolled in the Vanderbilt Main Heart Registry (VHVI) with NYHA class I-IV ischemic or non-ischemic cardiomyopathy (CM) (Figure 1). The study was approved by the Vanderbilt Institutional Review Board (IRB), and written consent was obtained from participants.

A retrospective medical chart review was performed to collect clinical data including serial measurements of LVEF from every documented 2D-Echo report with corresponding dates of

the studies included. LVEF was recorded as either an LVEF range or exact LVEF, depending on the interpretation. Cardiovascular risk factors were collected, including patient history of diabetes mellitus, hypertension, hyperlipidemia, family history of early heart disease, and tobacco use. In order to assess for major outcomes in a population with HF, cardiac transplant and/or advanced therapy-free survival were recorded in patients followed from January 2009 to December 2016, with primary endpoints qualified as either death from all causes, date of orthotopic heart transplantation, or date of ventricular assist device (LVAD) implantation. Confirmation of these dates was obtained through the electronic medical record and published obituaries.

Serum NRG was measured using DuoSet enzyme-linked immunoassay (ELISA) (R&D cat# DY377) following manufacturer's instructions as previously described (13). Serum samples were diluted 1:3 in phosphate-buffered saline. Detection antibody was diluted in 2% normal goat serum (Invitrogen: cat # PCN5000). Streptavidin-conjugated horse radish peroxidase as included with the DuoSet system was used for detection using substrate solution (Thermo Scientific 1-Step Ultra TMB-ELISA cat # 34028) for color development. Reactions were stopped at 10 minutes and absorbance read at 450nm using a spectrophotometric plate reader. The lowest and detection limits of the assay were 0.3 to 30 ng/ml. Samples with undetectable levels (n = 41) were assigned a value of 0.156 ng/ml, halfway between 0 and the lowest point on the standard curve (0.312 ng/ml). Samples with values above the highest point on the standard curve (n=9) were re-run at higher dilutions (1:5, 1:30, and 1:60) and assigned values if these fell below the highest point on the standard curve. Samples with values above the highest standard at 1:60 dilution were assigned a value of 629 ng/ml corresponding to the highest standard at this dilution. Samples above the highest standard (n=9) were excluded from further statistical analysis (Figure 1).

Descriptive statistics of baseline demographic and clinical characteristics were presented, including median values and lower quartile and upper quartile values for continuous variables with counts and percentages for categorical variables. Cardiovascular risk factors and clinical characteristics were then compared between patients with ischemic versus non-ischemic cardiomyopathy (CM) using the Wilcoxon test for continuous variables and Chi-square test for categorical variables. Serum NRG levels were compared between patients with symptomatic versus asymptomatic HF and ischemic versus non-ischemic CM using Wilcoxon or Kruskal-Wallis tests.

To examine the manner in which endogenous, baseline serum NRG levels influenced the predicted trajectory of LVEF over the course of 11 months, a multilevel model analysis was conducted. Follow-up time was treated as a level one predictor of LVEF over the course of 11 months. From there, regression analysis was performed on the slope of the aforementioned level one model using level two predictors, serum NRG values and median baseline LVEF (considered to be the last documented LVEF from 2-D echo reports prior to date of serum NRG blood draw). The regression coefficients assigned to the intercept and slope of the trajectory of LVEF with respect to serum NRG values were then used to interpret the impact of NRG values on the predicted LVEF values over time. The random intercept only model was reported based on clinical judgment.

Lastly, Cox regression model was used to assess the relationship between endogenous serum NRG levels and risk of death, cardiac transplantation, and LVAD implantation as primary endpoints during an 11-month follow-up period. This was adjusted for covariates including age, history of hypertension, diabetes mellitus, hyperlipidemia, obesity, tobacco use, NYHA classification, and positive family history for early-onset cardiovascular disease.

Results

Of the 239 patients enrolled and undergoing statistical analysis, 16 (6.7%) had no active symptoms of HF (NYHA Class I), 70 (29.3%) NYHA Class II, 127 (53.1%) NYHA Class III, and 26 (11%) NYHA Class IV with a mean age of 58 years $\pm$ 13 (Table 1). 173 (72%) were male, 66 (28%) were female. Cardiovascular risk factors within the study population included diabetes (38.5%), hypertension (63.6%), hyperlipidemia (65.7%), family history of early cardiovascular disease (38.1%), BMI > 30kg/m² (36.4%), and tobacco use (51%). 63% of patients had a history of ICD implantation and 13% of patients were status-post LVAD implantation.

Mean serum NRG in asymptomatic patients (n=86) was 13ng/ml and 22ng/ml in symptomatic patients (n=153). There was no significant difference in mean serum NRG in patients with asymptomatic versus symptomatic LVSD (p=0.53) (Table 2). There was no significant difference in mean serum NRG between patients with non-ischemic CM with baseline LVEF $\leq$ 25% (n=61), non-ischemic CM with baseline LVEF >25% (n=40), ischemic CM with baseline LVEF $\leq$ 25% (n=66), and ischemic CM with baseline LVEF >25% (n=64) (p=0.35).

There was no joint significant effect of baseline serum NRG on the intercept and slope of LVEF predicted trajectories (LRT=4.2, df=2, p=0.122). However, baseline serum NRG exhibited a marginally significant effect on the trajectory slope of LVEF within the initial 11 months following date of NRG measurement, in which a positive correlation was observed between predicted LVEF values and serum NRG (b=0.04 for every 10-unit increase in NRG, p=0.07) (Figure 2).

Of the 239 patients enrolled in the Vanderbilt Heart Failure Registry in 2009–2010, there were 83 (35%) reported deaths, 31 (13%) underwent LVAD implantation, and 19 (8%) were status-post orthotopic heart transplantation by December 2016. Cox regression analysis demonstrated that higher levels of serum NRG did not significantly predict risk of disease progression in the form of the aforementioned adverse events within one-year follow-up. A modest, but non-significant correlation was observed between increased levels of serum NRG and 11-month advanced therapy and transplant-free survival when NRG was treated as a continuous variable (Hazard Ratio=0.95 for every 10-unit increased in serum NRG, p=0.3) (Figure 3).

Discussion

These findings support a potential relationship between an elevation of serum NRG and predicted improvement in LVEF in patients with ischemic and non-ischemic CM, in which NRG exhibited a marginally significant effect on predicted LVEF over the course of 11

months from when NRG was first measured. Although our findings document a modest, but non-significant correlation between 11-month transplant and advanced therapy-free survival probability and NRG levels, there is no apparent serum NRG value that clearly predicts an improved survival.

Our observations correspond with several findings from previous animal studies and clinical trials regarding NRG and improvements in cardiac function. NRG has been shown to improve survival and echocardiographic parameters in mice with viral myocarditis, improve cardiac contractility and left ventricular end diastolic pressure in beagles paced at 230 beats/min for 3 weeks with subsequent HF, and improve cardiac function in ischemic model rats (12). More recently, administration of NRG isoform, glial growth factor 2 (GGF2) or cimaglermin alfa, in rat models with surgically-induced myocardial infarction (MI) demonstrated a dose-dependent increase in absolute LVEF by 10%, which confirms prior findings regarding the potential of NRG at enhancing contractile performance and impacting myocardial remodeling (14). Treatment with GGF2 among MI-induced swines also demonstrated improvements in LV cardiomyocyte mitochondria and resulted in less fibrosis with *in vitro* studies showing a reduction in myofibroblasts, suggesting that NRG's anti-fibrotic effects may prevent remodeling post-MI (15). The marginally significant effects of NRG on predicted LVEF trajectory represented in this study may also reflect the aforementioned observations *in vivo* and *in vitro*.

Furthermore, clinical trials have demonstrated both acute and chronic improvements in cardiac function and hemodynamics following administration of recombinant human NRG to patients with HF and LVSD. Phase II clinical trials involving the EGF-domain only fragment of NRG have demonstrated increases in LVEF with decreases in end systolic volume (ESV) and end diastolic volume (EDV) among patients with HF and improvements in hemodynamics (increase in cardiac output by 30%, decrease in systemic vascular resistance by 20%, and decrease in pulmonary artery wedge pressure by 30%) in patients with stable HF (10, 11, 16). Single dose administration of GGF2 in 40 patients with HF demonstrated a dose-dependent improvement in LVEF lasting 90 days following infusion with no adverse events leading to withdrawal of the study (9). These improvements in LVEF following administration of NRG in patients with HF also correspond with our observations regarding marginally significant improvements in LVEF trajectory with increasing NRG levels.

Likewise, we found that among patients with NYHA I-IV ischemic and non-ischemic CM, a positive but non-significant correlation was observed between survival probability and elevations of NRG values, which may be a reflection of cardioprotective mechanisms demonstrated *in vivo*. We also observed that NRG exhibited a marginally significant effect on predicted LVEF values over the course of 11 months, further contributing to the current understanding of the cardioactive growth factor role NRG may play in the setting of cardiac stress observed both *in vivo* and through clinical trials described above.

While Ky et al reported that patients within the highest quartile of NRG (NRG >8.6ng/ml) had a significantly increased risk of death or transplantation compared to those within the lowest quartile (NRG <3.4ng/ml), we did not observe a particular NRG value that clearly

predicted such risks. The mean concentration of serum measured in our study was 18.7 ng/ml, which differs from the mean concentration reported by Ky et al of 7.0ng/ml. Higher observed concentrations of serum NRG in this study compared to Ky et al may be reflective of variation in technique used to quantify NRG despite using similar assays, or perhaps the patient cohorts differed in terms of illness severity or acuity.

These data suggest that elevated serum NRG in patients with LVSD may be a beneficial and compensatory physiologic response as opposed to indicating a progression of HF pathophysiologic response. Ky et al noted that higher serum NRG was associated with increased risk of adverse events after four years of follow-up, but we observed a slight positive correlation between transplant and advanced therapy-free survival probability within 11 months, which could be explained by an acute, stress-induced release of NRG in the setting of ongoing cardiovascular damage. This phenomenon may be associated with downstream cardioprotective effects such as enhanced cardiac myocyte cell survival and reparative growth acutely. Little is known about the diagnostic and prognostic utility of serum NRG and only a handful of studies described above have quantified circulating serum levels of NRG in patients with heart failure. This study was unique in that it examined circulating levels of serum NRG (instead of administration of NRG) in an effort to assess the short-term, clinical manifestations (LVEF trajectory, survival probability) of its cardioprotective mechanisms within approximately 11 months of follow-up. Our findings add to current understandings of its role in cardiac repair and suggest that NRG may be a useful biomarker of cardiovascular disease severity, especially if serial measurements are implemented in future studies.

The study was performed retrospectively and thus has several inherent disadvantages. The relatively small size of the heterogeneous cohort with varying degrees of cardiomyopathy severity makes the comparison of small differences in serum values difficult. Statistical analysis also did not adjust for heart failure therapies. However, analysis was adjusted for baseline LVEF in order to account for improvements in systolic function over the course of 11 months following enrollment. Additionally, the measurement of LVEF was performed as is typically done in standard practice, and although quantitative, was not reported in the same fashion throughout the study time period. Furthermore, the measurement of serum NRG was only done on study entry and serial measurements potentially correlating with an improvement in LVEF were not done and this is an important future study that needs to be completed. It would be important to consider how other biomarkers compare to NRG in terms of important heart failure outcomes, especially in the acute setting. This may further elucidate the role of these endogenous markers on cardiac myocyte contractility, repair and damage during stress. Future studies may also juxtapose serum values of endogenous NRG with other inflammatory markers to compare improvements in systolic function and survival probability within the same patient populations.

Conclusion

Serum NRG can be quantified in patients with left ventricular systolic dysfunction and there is a potential relationship between serum NRG, LVEF, and survival probability. Our data suggest there is no apparent level of serum NRG that clearly predicts an improved survival.

However, these findings indicate the need to accurately define the physiologic role of serum NRG in order to investigate its cardioprotective mechanisms in the setting of left ventricular systolic dysfunction and heart failure.

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Figure 1.
Patient selection criteria

Figure 2.
Effect of Serum NRG on Predicted LVEF ($p=0.07$)

Figure 3.
11-month Transplant and Advanced Therapy-Free Survival Probability versus Serum NRG
as Continuous Variable ($p=0.25$)

Table 1.

Descriptive data for NRG study population

Characteristic	Entire Cohort	Ischemic CM	Non-ischemic CM	p-value
	<i>n</i> =239	<i>n</i> =135	<i>n</i> =104	
Age (years) ³	58±13	61±11	54±15	<0.001 ¹
Gender				
Male	173 (72)	107 (79)	66 (64)	0.007 ²
Female	66 (28)	28 (21)	38 (36)	
Diabetes	92 (38)	68 (50)	24 (23)	<0.001 ²
Hypertension	152 (64)	94 (70)	58 (56)	0.03 ²
Hyperlipidemia	157 (66)	111 (82)	46 (44)	<0.001 ²
Family history of CAD	91 (38)	64 (47)	27 (26)	<0.001 ²
BMI >30kg/m ²	87 (36)	48 (36)	39 (38)	0.8 ²
Tobacco use	122 (51)	84 (62)	38 (36)	<0.001 ²
ICD	150 (63)	82 (61)	68 (65)	0.5 ²
LVAD	32 (13)	20 (15)	12 (12)	0.5 ²
NYHA Class				0.9 ²
I	16 (7)	8 (6)	8 (8)	
II	70 (29)	39 (29)	31 (30)	
III	127 (53)	73 (54)	54 (52)	
IV	26 (11)	15 (11)	11 (11)	
Serum NRG-1β (ng/ml) ⁴	18.7±43.9 (2.3, 0.2–15.4)	11.9±30.2 (2.3, 0.2–10.0)	27.7±56.1 (2.9, 0.2–30.1)	0.2 ¹

All data except serum NRG and age abbreviated as *n*(percent of total)¹Wilcoxon test²Pearson test³Mean±Standard Deviation⁴Mean±Standard Deviation (Median, lower quartile-upper quartile); CM=Cardiomyopathy, BMI=Body Mass Index, ICD=Implantable Cardioverter-Defibrillator, LVAD=Left Ventricular Assist Device, NYHA=New York Heart Association.

Table 2:

Serum NRG values by HF status and Cardiomyopathy type

	Mean ¹ ±SD (Median, lower quartile-upper quartile)	<i>p</i> -value
Asymptomatic HF (n=86)	13.0±25.2 (2.2, 0.2–10.8)	0.53 ²
Symptomatic HF (n=153)	22.0±51.4 (2.7, 0.2–16.5)	
Non-ischemic CM LVEF 25% (n=61) *	31.6±60.3 (4.0, 0.2–29.9)	0.35 ³
Non-ischemic CM LVEF >25% (n=40) *	23.7±51.1 (1.9, 0.2–30.9)	
Ischemic CM LVEF 25% (n=66) *	12.8±37.2 (2.3, 0.2–12.3)	
Ischemic CM LVEF >25% (n=64) *	10.1±20.6 (1.8, 0.2–6.8)	

¹ (ng/ml)² Wilcoxon test³ Kruskal-Wallis test

* Baseline LVEF; HF=Heart Failure, CM=Cardiomyopathy, LVEF=Left Ventricular Ejection Fraction.